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Development of a Cost-Effective Culture Medium for the Bacterial Cellulose Production Using Food Industry Wastes

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Abstract

Background and Objective: Use of bacterial cellulose has been interested in various industries, especially medical and pharmaceutical industries, due to its unique characteristics, compared to plant cellulose. However, bacterial cellulose production costs have limited its industrial uses, compared to plant cellulose. Decreasing costs of the culture media is one of the effective parameters for the industrial production of bacterial cellulose. This is the first report on combination of vinasse and glucose syrup as a bacterial cellulose culture medium.

Material and Methods: Two inexpensive culture media were developed for high-level production of bacterial cellulose based on food industrial wastes of corn steep liquor-glucose and vinasse-glucose syrups. Concentrations of glucose syrup and corn steep liquor as a culture medium and concentrations of vinasse and glucose syrup as another culture medium were optimized using response surface method with central composite design to maximize bacterial cellulose production yields.

Results and Conclusion: Under the optimal conditions after seven days, 14.8 and 13.3 $g.l^{-1}$ dry bacterial cellulose were achieved in corn steep liquor-glucose syrup and vinasse-glucose syrup respectively. Yield of produced bacterial cellulose from these two cost-effective culture media was one of the highest values reported for bacterial cellulose. Furthermore, the produced bacterial cellulose was characterized using Fourier-transform infrared spectroscopy, X-ray diffraction and scanning electron microscopy. The two culture media in this study included significant bacterial cellulose production yields, compared to similar food industrial waste culture media.

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1. Introduction

Due to bacterial celloluse (BC) characteristics such as crystallinity, degree of polymerization, high strength and purity, BC is used in various industries, especially in medical industries as skin and artificial veins, dressings and sanitary napkins, drug delivery systems and cell scaffolds in tissue engineering. Moreover, it has been used in food industries as well [1-3]. Despite genetic manipulations and development of novel cultivation methods to increase production yields and decrease production costs, mass production of BC needs further investigations [4,5]. Recently, various BC strains, inexpensive culture media and supplementary materials have been investigated for the mass production of BC [5-7]. Fermentation culture media costs for BC production, especially on a scale-up, are significant. These costs are reported nearly 30% of total costs of BC production, which is a big challenge [8,9]. Usually, Hestrin-Schramm (HS) culture medium is used in laboratories to produce BC [10]. Babeaipour et al. have used food industrial wastes to produce BC, decrease the production costs and increasing the yields of BC production [11,12]. Corn steep liquor (CSL) mostly consists of protein and lactic acid and small quantities of carbohydrates and fats. This substance, which is provided by

the food industries, contains corn and forms appropriate culture media with rich nutrients [13,14]. Reports on increasing the yields of BC production to 90% with culture media containing CSL have been published by Cheng et al. [15]. Based on another report, adding 8% of CSL to the culture media increased BC production by 47% [16]. A mixture of 4% glucose and fructose with 10% CSL increased BC production nearly three times, compared to its production with HS media [17]. The CSL has been used in culture media for several decades to increase production of various biological products. Moreover, vinasse has been used as an appropriate nitrogen source for the production of biological derivatives. However, a few studies have assessed these compounds for BC production in optimal culture media.

Vinasse is a concentrated solution from the anaerobic sugar fermentation to produce ethanol, which produces 10-15 times more than ethanol as a waste. Vinasse is a watery compound containing minerals and organic elements such as nitrogen, phosphorus and potassium. Various results have been reported on use of vinasse as a culture medium for BC production. Barshan et al. have introduced vinasse as an inexpensive culture medium that simultaneously increases BC production by more than 30%, compared to HS culture. Based on a study by Barshan et al., HS culture media included higher BC production yields for cellulose production than that vinasse did [18]. This decrease in production yields includes imbalances between sugar and nitrogen in the culture media and pH changes [18]. Vinasse includes acidic pH, high chemical oxygen demand, high volatile solids and total solid levels [19]. Vinasse from various ethanol fermentation industries includes various compositions [20]. Several raw materials have been used to produce ethanol, giving unique characteristics to each produced vinasse. These raw materials include sugar products (sugarcane, sugar beet, molasses and sweet sorghum), starch products (corn, wheat, rice, cassava and barley), cellulosic materials (harvest residues, bagasse and wood), fruit sources and agave [20].

In the present study, two culture media from food industrial wastes and fermented ethanol, including CSL-glucose and vinasse-glucose syrups, were used to produce a cost-effective culture medium for high BC production. Proportions of compounds of these cultures were assessed and optimized based on BC production yields within seven days. This study was an optimization process of culture media components with the response surface method and central composite design methodology. Moreover, BCs from the two culture media were compared regarding production yields and appearance characteristics.

2. Materials and Methods

2.1. Microorganism and culture medium

Acetobacter xylinum BPR2001 (*Acetobacter xylinum sucrofermentas*) from the Microbial Bank of Japan produced the BC. This strain was selected because it included significant BC production yields, compared to other BC producing strains. The HS medium was used as an inoculum culture medium. Glucose syrup, CSL from Zar Macaron, Iran, and vinasse from Bidistan, Iran, were used for BC production. Components and preparation conditions of each culture medium are detailed as follows.

2.2. Cultivation conditions

The cell bank was purchased as a colony form. Therefore, a colony was transferred to the liquid culture media and passaged. Homogenous media from the culture with appropriate BC production were separated into 1-ml vials and transferred to the cell bank for further use. To prepare the inocula, 5-10 ml of the prepared cell bank were transferred to 50 ml of HS culture media. Inocula were preserved in static conditions at 30 °C for two days. The HS culture media were prepared according to Rouhi et al. [13]. The 5 ml inocula were transferred to 50 ml of glucose syrup and CSL culture media. Similarly, 50 ml of glucose syrup and vinasse solution were inoculated and incubated at 30 °C for seven days. After culturing BC, the gelatin film was washed and its wet and dry weights were recorded. All experiments were carried out under sterile conditions.

2.3. Bacterial cellulose purification

After the cultivation of BC, the resulting gelatin film was not pure and contained bacteria or residues of the culture media. A process for BC purification included treatments with substances such as alkali or acidic solutions and hot water. These purification steps could be used alone or in combination with each other. In this study, BC was boiled at 100 °C for 30 min in 1 N NaOH solution. Then, cellulose bacteria were washed using distilled water (DW) to remove the remaining soda until pH of the solution from the cellulose bacteria was neutralized [9,21,22].

2.4. Production yields of the bacterial cellulose

Weight of the purified wet gelatin film was measured after removing excess water using filter papers. Then, complete dry weight of BC was measured after samples were incubated at 70 °C for 24 h using oven. Dry weight of BC was measured as well.

2.5. Optimization of bacterial cellulose production

Briefly, BC productions in the two culture media (glucose syrup-CSL and glucose syrup-vinasse) were investigated at five levels using response surface method and central composite design algorithm. Table 1 shows concentrations of the components of BC culture media (glucose syrup-CSL and glucose syrup-vinasse) suggested by central composite design. In this design, factor A was the concentration of glucose syrup in the two culture media, B was the concentration of CSL and vinasse, separately. Results included production yields within seven days. Experiments were carried out under similar conditions using 250-ml Erlenmeyer flasks.

2.6. Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy (FT-IR) spectra were recorded using Perkin-Elmer spectrophotometer (Spectrum two model, Perkin-Elmer, Beaconsfield, UK) operating in 400-4000 cm^{-1} at 2 cm^{-1} resolution to study chemical structures of the samples regarding their contributing functional groups.

2.7. Scanning electron microscope

Morphology of the dried samples was studied using scanning electron microscopy (SEM) (Tuscan s.r.o. Brno, Czech Republic). Dried pieces were coated with gold and studied by SEM. The accelerated voltage included 40 kV.

2.8. X-ray diffraction

Dried samples were hand grinded using mortar and pestle and then studied using X-ray diffraction (XRD) technique (D8 Advance instrument, Bruker, Germany) with a CuKα radiation source. Briefly, 40-kV operating voltage and 30-mA electric current were used on samples. Samples were studied within a 5°-90° range and a 3° min^{-1} speed rate. Crystallinity index was calculated according to Eq. 1 based on a method previously reported by Segal [23]:

$$\text{Crystallinity index (\%)} = \frac{I_{200} - I_{am}}{I_{200}} \times 100 \qquad \text{Eq. 1}$$

Where, I_{200} was intensity of the diffraction peak assigned to the plane (200) at $2\theta = 22.7°$ for crystalline cellulose (cellulose I) and I_{am} was intensity of the diffraction signal at $2\theta = 18°$ coming from the amorphous part of the sample.

3. Results and Discussion

Quantity of BC produced by *A. xylinium* in these optimized culture media was significant, compared to similar studies such as the study of Wu, Kumar and Rani, which used wastewater from rice wine distillery, tomato juice, and coffee cherry husk-CSL as culture media [24-27]. Wet weight of the samples was in the range of 580.84-540.96 $g.l^{-1}$. Based on Table 1, dry weight of the samples was in 5.5-13.1 $g.l^{-1}$ range for CSL culture and 5-15 $g.l^{-1}$ for vinasse culture. This increase in production of CSL could be linked to differences in quantities of nutrients in CSL, compared to vinasse culture media. As previously stated, CSL mostly contains crude proteins, amino acids, minerals, vitamins (especially vitamin B), reducing sugars and organic acids. Source of vinasse used in this study was sugar beet, which contained potassium, sulfur, phosphorus, nitrogen, calcium and magnesium. Assessment of exact quantities of vinasse and CSL components is difficult because these culture media are complex. However, existence of the highlighted nutrients has been verified by the source producers. Differences in BC production in these two culture media could be linked to various C: N ratios. The ratio was 10 for the optimal CSL-glucose syrup culture media and 6 for the optimal vinasse-glucose syrup culture media. Figure 1 shows that more than 90% of the culture media were converted to BC after adjusting the optimal concentration of CSL and glucose syrup.

3.1. Optimization of bacterial cellulose production

Based on the ANOVA Table (Table 2), *p*-value and F-value were significant values. Design of experts suggested selecting specific and practical factors with a probability of 95%. Based on Table 2, sum of squares, mean squares and F-values, which indicated ability of the models to decrease the fluctuation, results for models (BC production yields) were acceptable. The *p*-values less than 0.05 were statistically significant. Total *p*-values of the two models were≤0.0001, which indicated significance and stability of the models. Based on the *p*-values and F-values of the parameters, importance of glucose concentration was less than that of CSL and vinasse concentrations (Table 2). Insignificancy of lack of fitness term was appropriate as well. Higher R-squared (more than 95%) indicated the model accuracy in describing the process

In culture media containing CSL, R-squared was 0.9826 and in vinasse, the value was 0.9861 (Table 3), which indicated matching the model to the results. Predicted R-squared reflected appropriately of the model for predicting other data, which showed reasonable and appropriate values for the discussed models. Differences between the adjusted R-squared and the predicted R-squared were less than 0.02 in the two models, which revealed correctness and reliability of the models designed based on the experimental data. Adeq precision demonstrated the signal-to-noise ratios, which were 23.9493 and 20.4093 in culture media containing CSL and vinasse, respectively. These values showed that the model could be used to predict results in design space areas as they were greater than 4. Hence, the final equation of these models was achieved using Eqs. 2 & 3. Eq. 2 was a result of the design of culture media containing CSL and Eq. 3 belonged to culture media containing vinasse.

$$R1 = 3.79 - 0.5468A + 3.12B - 1.36A^2 - .71B^2 \qquad \text{Eq. (2)}$$

$$R1 = 12.60 + 0.8877A + 1.48B + 0.5500AB - 2.49A^2 - 2.54B^2 \qquad \text{Eq. (3)}$$

The contour plots (Figs. 2 and 3) show interactions of the two factors and the optimum of each one for the maximum response. Data in Fig. 2a shows that increasing the concentration of CSL led to increases in the production yields of BC. One reason for this increase could include the presence of lactate in CSL. Based on the report by Amiri et al., CSL is an appropriate source that provides carbon, nitrogen, amino acids and vitamins for bacteria [28].

Table 1. Component concentrations of the culture media and dry weight production yields as responses

Culture medium of Glucose syrup and CSL					Culture medium of Glucose syrup and vinasse				
Std	Run	Factor 1 A:Glucose syrup ($g.l^{-1}$)	Factor 2 B: CSL ($g.l^{-1}$)	Response R	Std	Run	Factor 1 A: Glucose syrup ($g.l^{-1}$)	Factor 2 B: Vinasse ($g.l^{-1}$)	Response R
4	1	50.0	100.0	13.3	11	1	26.0	21.5	12.6
6	2	56.2	75.0	10.1	5	2	6.2	21.5	6.0
2	3	50.0	50.0	7.3	2	3	40.0	8.0	6.3
11	4	35.0	75.0	13.8	9	4	26.0	21.5	13.1
9	5	35.0	75.0	12.9	1	5	12.0	8.0	5.9
7	6	35.0	39.6	5.8	6	6	45.8	21.5	8.9
1	7	20.0	50.0	8.1	8	7	26.0	40.6	9.2
10	8	35.0	75.0	14.6	10	8	26.0	21.5	12.1
2	9	35.0	110.3	15.0	4	9	40.0	35.0	10.7
3	10	20.0	100.0	14.0	3	10	12.0	35.0	8.1
5	11	13.8	75.0	12.0	7	11	26.0	2.4	5.5

Table 2. Analysis of variance for the assessment of the effects of culture media on the production of bacterial cellulose using response surface method

Glucose syrup-CSL culture medium						
Source	Some of squares	df	Mean square	F-value	P-value	
Model	101.24	4	25.31	84.80	< 0.0001	significant
A-glucose	2.39	1	2.39	8.01	0.0299	
B-CSL	77.82	1	77.82	260.71	< 0.0001	
AB	2.20	1	2.20	4.11		
A^2	10.45	1	10.45	35.03	0.0010	
B^2	16.48	1	16.48	55.20	0.0003	
Residual	1.79	6	0.2985			
Lack of Fit	0.3459	4	0.0865	0.1197	0.9627	not significant
Pure Error	1.45	2	0.7225			
Cor Total	103.3	10				
Glucose syrup -vinasse culture medium						
Source	Some of squares	df	Mean square	F-value	P-values	
Model	80.12	5	16.02	70.88	0.0001	significant
A- glucose	6.30	1	6.30	27.88	0.0032	
B-vinasse	17.50	1	17.50	77.42	0.0003	
AB	1.21	1	1.21	5.35	0.0686	
A^2	34.94	1	34.94	154.57	< 0.0001	
B^2	36.36	1	36.36	160.84	< 0.0001	
Residual	1.13	5	0.2261			

Table 3. Fit statistics for the assessment of the effects of culture media on the production of bacterial cellulose using response surface method

The glucose syrup-CSL culture medium				The glucose syrup-vinasse culture medium			
Std. Dev.	0.5463	R^2	0.9826	Std. Dev.	0.4755	R^2	0.9861
Mean	11.56	Adjusted R^2	0.9710	Mean	8.95	Adjusted R^2	0.9722
C.V. %	4.73	Predicted R^2	0.9522	C.V. %	5.32	Predicted R^2	0.9310
		Adeq Precision	23.9493			Adeq Precision	20.4093

Acetobacter strains are reported to oxidize glucose and produce gluconate. By increasing the glucose concentration in culture media, this oxidation reaction leads to decreases in pH and thus inhibits cell growth and cellulose production. Based on Fig. 2a, concentrations higher than the reported range of glucose syrup might lead to the inhibition of bacterial growth and BC production [29]. Presence of CSL decreases this inhibition and creates various metabolites in CSL that prevent overproduction of gluconic acid. Gluconic acid production can be decreased by other primary carbon sources such as sucrose or glycerol or additives such as ethanol, which serves as an additional source of ATP for BC production. For example, culture media such as molasses and beer fermentation wastes may include high potentials for BC production due to the presence of sucrose and ethanol, respectively.

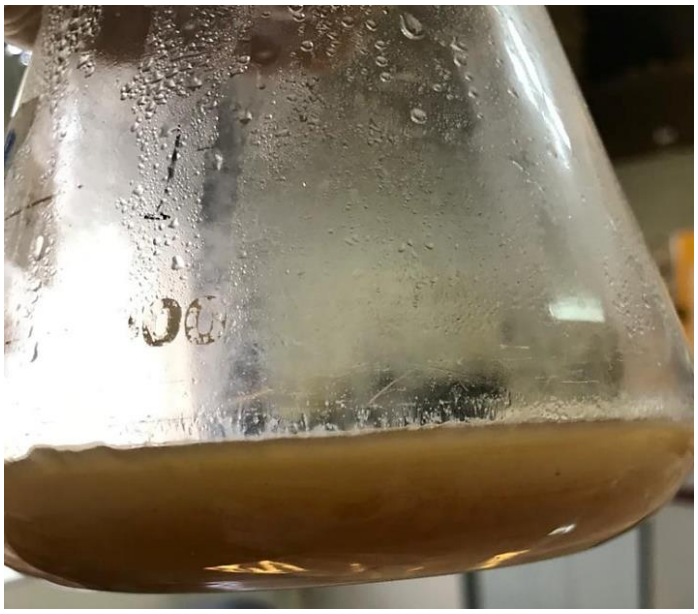

Figure 1. Bacterial cellulose optimal cultivation with more than 90% conversion of the culture media to the product

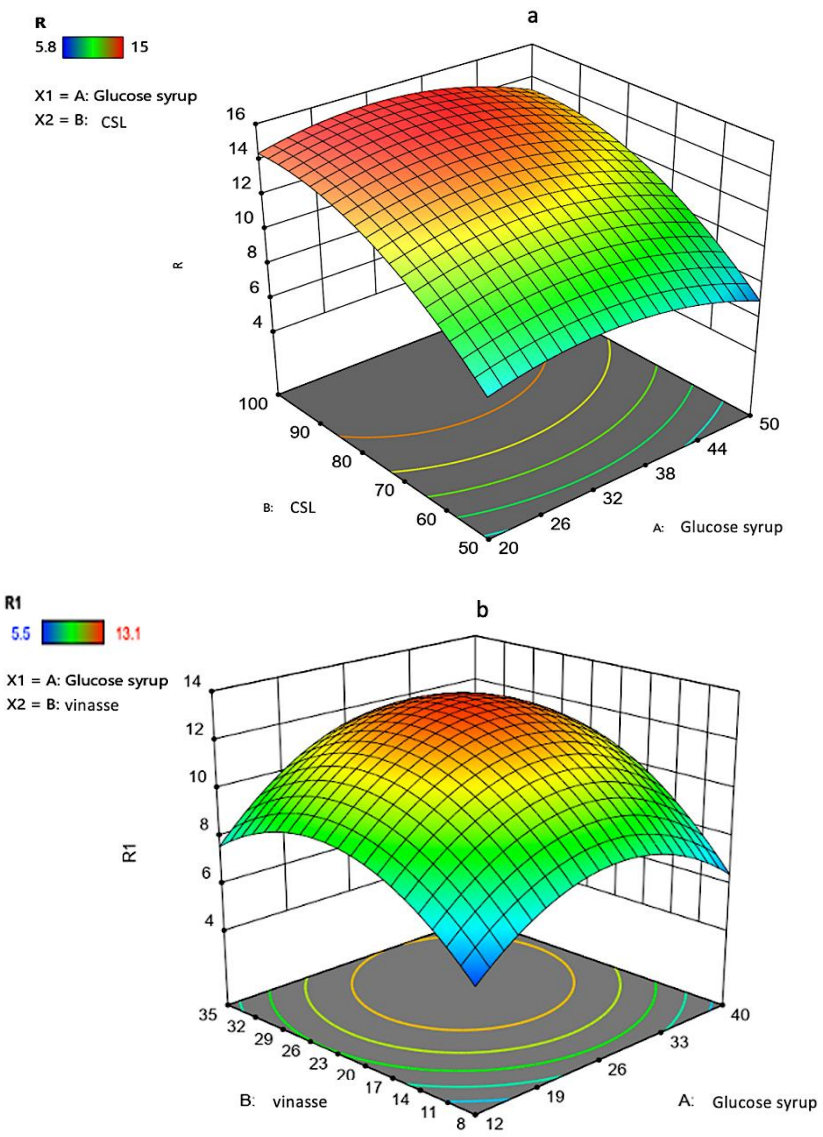

Figure 2. Effects of a) glucose syrup and CSL concentrations and b) glucose syrup and vinasse concentrations on BC production yields

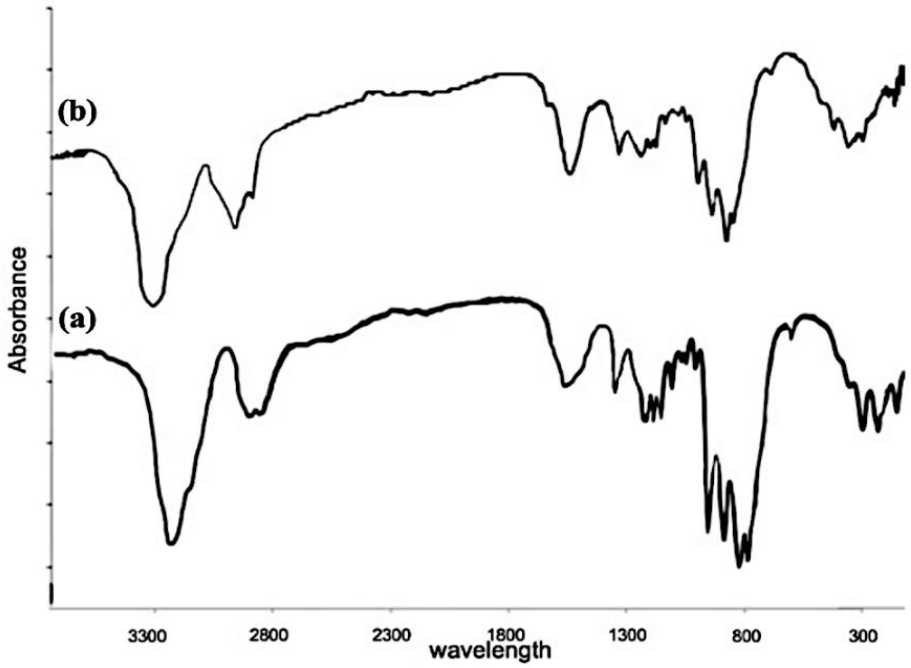

Figure 3. Fourier transform infrared spectroscopy spectra of a) bacterial cellulose from CSL-glucose syrup and b) bacterial cellulose from vinasse-glucose syrup

Figure 2b shows that increases in the quantity of vinasse and glucose syrup to a certain value led to increases in BC production yields and further increases in concentrations of these substances led to decreases in BC production. Glycerol, ethanol, organic acid and residual sugars have been identified in vinasse and can act as parallel energy sources that do not need complete oxidation of glucose to gluconic acid to provide energy [30,31]. Thus, one of the reasons for decreases in BC production yields at high concentrations of vinasse could include increases in pH, other than appropriate quantities for BC production [30]. Under optimal conditions, pH of the culture media on Days 1, 3 and 7 for the culture media containing vinasse respectively included 6.1, 5.7 and 5.9 and for culture media containing CSL included 6, 4.7 and 5.2, respectively. The optimum pH of 5 for BC production by *A. xylinum* was reported by Zahan et al. [32]. In general, vinasse includes high potentials to increase BC production. Dilution of the substance and addition of other components to vinasse-based culture media seem necessary because previous studies using vinasse alone resulted in a little or no increases in the production yields [19]. Supplementing vinasse with components such as an appropriate carbon source, which provides the carbon-nitrogen balance necessary for the BC production, can help increase the production yields. Heydon et al. supplemented the reaction with beet molasses, vinasse and WBFB streams as sources of carbon and nitrogen [29]. In the present study, BC production yield in culture media containing CSL-glucose syrup was higher than that in vinasse-glucose syrup. At optimal concentrations suggested by the central composite design (105.3 $g.l^{-1}$ CSL and 34.1 $g.l^{-1}$ glucose syrup), 14.8 $g.l^{-1}$ BC were achieved. Optimal concentrations for the culture media containing vinasse were 23.9 $g.l^{-1}$ vinasse and 27.5 $g.l^{-1}$ glucose syrup, which under experimental conditions led to the production of 13.3 $g.l^{-1}$ substance. This production quantity under static conditions was significant, compared to those from previous studies [32,33]. The culture media cost was approximately 50-60% decreased using CSL-glucose syrup and vinasse-glucose syrup culture media, respectively, compared to HS culture media. Moreover, production yields of these culture media were significant, compared to that of HS culture media. Therefore, it can be concluded that the suggested culture medium, prepared from food industrial wastes, is cost-effective.

3.2. Characterization analysis

Figure 3 shows particular differences in FTIR spectra of the samples. Almost all the peaks appeared in similar positions. Therefore, results for BC from the two culture media (CSL-glucose syrup and vinasse-glucose syrup) have shown similar chemical bonds. Naturally, intensity of this peak depends on the intermolecular hydrogen bonds. In Fig. 3, increases in 3000–3600 cm^{-1} range were linked to O-H stretching forces in BC. The peak near 3250 cm^{-1} in Fig. 3a was associated to cellulose Ia which decreased in Fig. 3b, indicating decreased intermolecular hydrogen bonds. This result might show tighter structures of the cellulose from culture media containing CSL, compared to culture media containing vinasse. Technically, C-H stretching can be attributed to the peak near 2800-2900 cm^{-1}. Other bands in the range 1100-850 cm^{-1} were linked to C-O-C and C-C vibration bonds of glycosidic bonds and pyranoid rings. Bonds near 1553 and 1350 cm^{-1} were dedicated to the

stretching of the carboxyl group and -CH_2, respectively and O-H in-plane bending was nearly 1318 cm^{-1} [34-37].

Figure 4 shows XRD patterns of the BC samples from culture media containing CSL and vinasse. As expected, the highest diffraction intensity was observed at 2θ of 14.5°, 16.7° and 22.7 °, corresponding to 100, 010 and 110 crystal planes of cellulose Iα, respectively. Diffraction peaks of the two samples were almost similar and included the crystal structure of cellulose Iα . However, shifts at 14.5° towards a higher intensity and that at 16.4° towards a lower intensity were regarded as results of the underlying monoclinic Iβ phase [Iβ = (1-10) and (110), respectively] that could coexist. Crystallinity assessed via Segal method was similar to that of previous studies as values of 76.5 and 70.7% were reported for culture media containing CSL (Fig. 4a) and vinasse (Fig. 4b), respectively. Crystals in the current study supported findings of previous studies, revealing that the culture media and certain additives could affect formation of cellulose crystals [38,39]. Differences in the crystallinity index of BC produced from various culture media were due to the hydrogen bonds of various strengths between the cellulose chains.

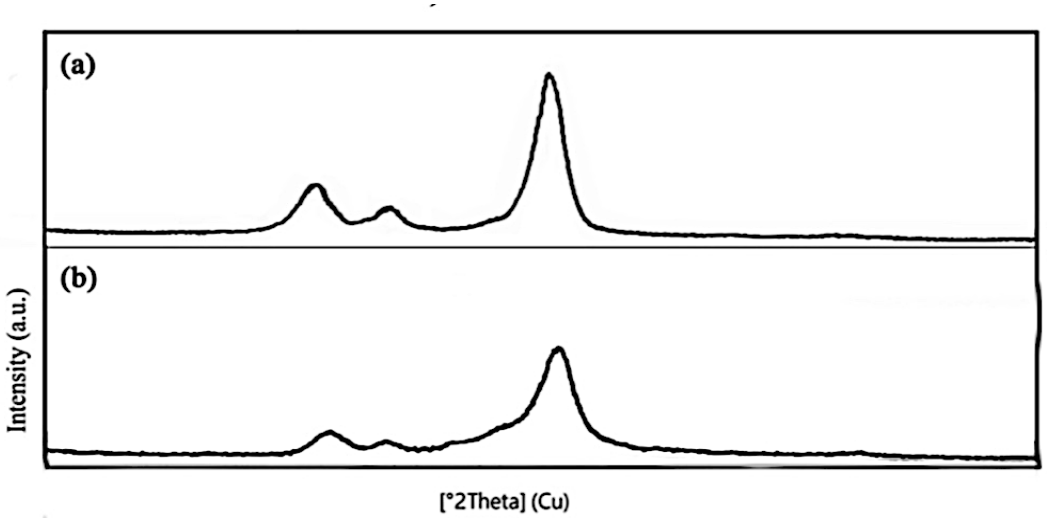

Figure 4. X-ray diffraction patterns of a) bacterial cellulose from CSL-glucose syrup and b) bacterial cellulose from vinasse-glucose syrup

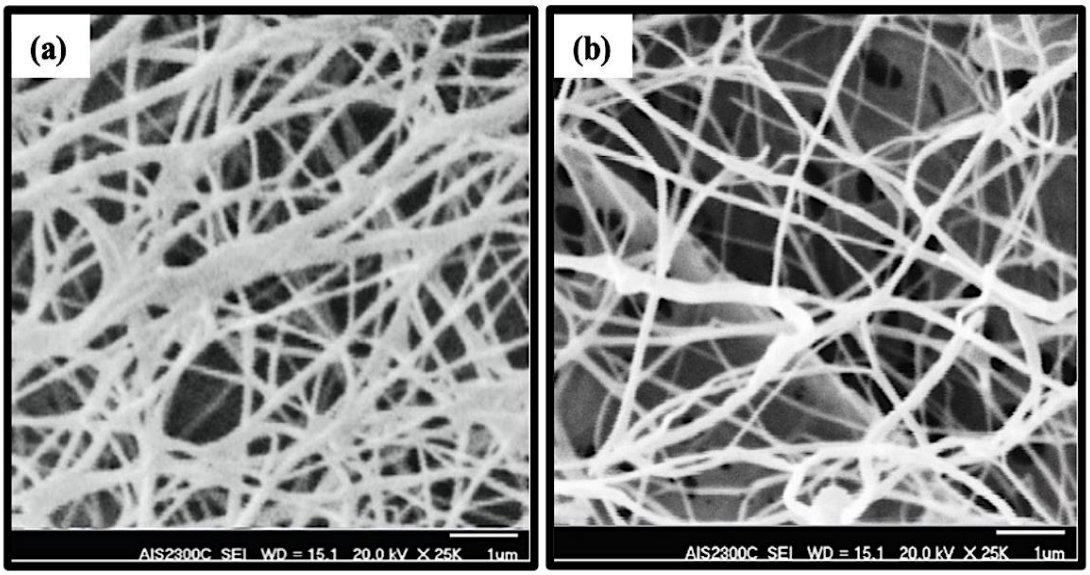

Figure 5. Scanning electron microscopy images of a) bacterial cellulose from CSL-glucose syrup and b) bacterial cellulose from vinasse-glucose syrup

The SEM of samples from the two culture media was investigated (Figs. 5a, b). Fibers in the two images were distributed in the matrix. Moreover, BC from culture media containing vinasse showed further porous structures, compared to other samples (Fig. 5a). Diameter of the cellulose fibers from the vinasse culture was smaller than that of the cellulose fibers from the CSL culture. These morphological differences could be due to differences in the metabolism process of nutrient consumption and cellulose production.

4. Conclusion

This study investigated effects of cost-effective culture media on production yields of BC and its characteristics. One of the most significant parameters of BC production is the concentration of culture media components and their types. In this study, culture media were optimized using central composite design to maximize the production yields of BC. Glucose syrup was used as the carbon source and CSL and vinasse as the nitrogen sources in the culture media. The CSL as a nitrogen source was more effective in increasing yields of BC production and the crystalline index of BC from the culture media was higher than that from culture media containing vinasse. In the optimized CSL-glucose syrup and vinasse-glucose syrup culture media, 14.8 and 13.3 $g.L^{-1}$ BC were achieved, respectively. These results have suggested that CSL and vinasse can be low-cost substrates for BC production without expensive nitrogen supplementations such as yeast extract and peptone. Based on the results, total price of the two culture media was much lower than that of laboratory ones such as HS. The 50% CSL-glucose syrup and 60% vinasse-glucose syrup decreased the cost of culture media, compared to that the HS did. However, CSL included a higher BC production yield and hence was preferred. Cellulose from culture media containing CSL included stronger hydrogen bonds, higher crystallinity index and denser network than those the cellulose from culture medium containing vinasse did. These results were generated using characterization of FTIR and XRD patterns and SEM analyses, respectively.

5. Acknowledgements

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6. Conflict of Interest

The authors report no conflict of interest.

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توسعه یک محیط کشت مقرون به صرفه برای تولید سلولز باکتریایی با استفاده از ضایعات صنایع غذایی

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چکیده

سابقه و هدف: استفاده از سلولز باکتریایی به‌دلیل ویژگی‌های منحصر به‌فرد آن در مقایسه با سلولز گیاهی در صنایع گوناگون به‌ویژه صنایع پزشکی و دارویی مورد توجه قرار گرفته است. با این‌حال، هزینه‌های تولید سلولز باکتریایی، کاربردهای صنعتی آن را در مقایسه با سلولز گیاهی محدود کرده است. کاهش هزینه‌های محیط کشت یکی از عوامل موثر برای تولید صنعتی سلولز باکتریایی است. این اولین گزارش درخصوص ترکیب شربت وینلس و گلوکز به‌عنوان یک محیط کشت سلولز باکتریایی است.

مواد و روش ها: برای تولید سلولز باکتریایی در مقیاس بالا و برپایه ضایعات صنعت غذایی ذرت، دو محیط کشت ارزان‌قیمت، یعنی شربت گلوکز- مایع خیسانده ذرت و همچنین وینلس- شربت گلوکز تهیه شد. غلظت شربت گلوکز-مایع خیسانده ذرت به عنوان یک محیط کشت و غلظت شربت گلوکز- وینلس به عنوان محیط کشت دیگر با استفاده از روش سطح پاسخ با طرح مرکب مرکزی، به‌منظور رسیدن به بیشترین بازده تولید سلولز باکتریایی بهینه سازی شد.

یافته‌ها و نتیجه‌گیری: در شرایط بهینه پس از هفت روز، در محیط کشت شربت گلوکز - مایع خیسانده ذرت و شربت گلوکز-وینلس به‌ترتیب $g.l^{-1}$ ۱۴/۸/ و ۱۳/۳ سلولز خشک باکتریایی به‌دست آمد. علاوه بر این، سلولز باکتریایی تولید شده با استفاده از طیف‌سنجی فروسرخ تبدیل فوریه[۱]، پراکنش پرتو ایکس[۲] و میکروسکوپ الکترونی روبشی[۳] مشخص شد. در این مطالعه، دو محیط کشت از لحاظ راندمان تولید سلولز باکتریایی مشابه محیط کشت ضایعات صنایع غذایی بودند.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.

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- سلولز باکتریایی
- مایع حاصل از مرحله خیساندن ذرت
- شربت گلوکز
- بهینه‌سازی
- وینلس

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